

IN THE CLAIMS

Please replace the currently pending claims with the following amended claims. In accordance with the newly instituted revised amendment format, a complete set of claims follows, with new material to be added to the claims shown as underlined, and material to be deleted shown as ~~struck through~~; no clean copy of the claims need be provided.

1. (Previously amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide in said cells comprising said intracellular analyte;
- c) contacting said cells with a medium comprising a chaotropic agent to wash said cells;
- d) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby cells comprising said intracellular analyte are specifically labeled; and
- e) detecting a signal from cells comprising said detectable label using a flow cytometric device, wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

2. (Twice amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide conjugated to a detectable label in said cells comprising said intracellular analyte, whereby cells comprising said intracellular analyte are specifically labeled; and

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c) contacting said cells with a medium comprising a chaotropic agent to wash said cells;

d) detecting a signal from cells comprising said detectable label using a flow cytometric device, wherein the presence of said signal is correlated to the presence of said intracellular analyte in said cells.

3. (Previously amended) The method of claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

4. (Previously amended) The method of claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

5. (Previously amended) The method of claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme;

(ii) removing unbound binding partner from said cells; and

(iii) contacting bound binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

6. (Previously amended) The method of claim 1 or 2, wherein said detectable label is a fluorochrome.

7. (Twice amended) The method of claim 6, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridinin chlorophyll, and cyanine.

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8. (Previously amended) The method of claim 5, wherein said enzyme is selected from the group consisting of hydrolysases, peroxidases, oxidase, esterases, glycosidases and phosphatases.

9. (Previously amended) The method of claim 5 wherein said enzyme is horseradish peroxidase.

10. (Twice amended) The method of claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a first binding ~~parts~~ partner that specifically binds to said analyte, and a second binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme ~~the deposition of tyramide~~;

(ii) removing unbound second binding partner from said cells; and

(iii) contacting bound second binding partner with tyramide and said substrate for said enzyme, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

11. (Previously amended) The method of claim 10, wherein said second binding partner is an immunoglobulin-enzyme conjugate.

12. (Previously amended) The method of claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

13. (Previously amended) The method of claim 12, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

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14. (Previously amended) The method of claim 1 or 2 wherein, said one or more cells are cultured cells.

15. (Previously amended) The method of claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organeller antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.

16. (Previously amended) The method of claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.

17. (Previously amended) The method of claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.

18. (Previously amended) The method of claim 1 or 2, wherein said one or more cells are obtained from a patient.

19. (Previously amended) The method of claim 18, wherein said signal is correlated to a diagnosis of a disease in said patient.

20. (Previously amended) A kit for performing a method according to claims 1 or 2, wherein said kit comprises a medium comprising a chaotropic agent; an analyte-specific binding partner conjugated to an enzyme that catalyzes the deposition of tyramide in the presence of tyramide and a substrate for said enzyme; a substrate for said enzyme; and a tyramide reagent selected from the group consisting of unlabeled tyramide and tyramide conjugated to a detectable label, wherein if said tyramide reagent is unlabeled tyramide, said kit further comprises a tyramide-specific binding partner conjugated to a detectable label.

37. (Previously added) The method of claim 1 or 2, wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.